

The University of Chicago

GROWTH AND MORPHOLOGY
OF TOBACCO TISSUE CULTURES
IN VITRO

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF BOTANY
1947

By
SAMUEL M. CAPLIN

Reprinted from
THE BOTANICAL GAZETTE
Vol. 108, No. 3, March, 1947

The University of Chicago

GROWTH AND MORPHOLOGY
OF TOBACCO TISSUE CULTURES
IN VITRO

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF BOTANY
1947

By
SAMUEL M. CAPLIN



Reprinted from
THE BOTANICAL GAZETTE
Vol. 108, No. 3, March, 1947

GROWTH AND MORPHOLOGY OF TOBACCO TISSUE CULTURES IN VITRO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 580

SAMUEL M. CAPLIN

Introduction

A milestone in botanical research was reached in 1939, when WHITE (22), using stem tissue from tobacco, and GAUTHERET (4) and NOBECOURT (13), using cambial tissue from carrot root, showed independently that excised plant tissues will grow indefinitely if they are repeatedly subcultured to fresh nutrient of the proper constitution. After HABERLANDT (8) had formulated the idea of a plant tissue culture at the beginning of the century, the first big step was made

when ROBBINS (15, 16, 17) and KOTTE (11, 12) attempted independently to grow excised root tips and stem tips in nutrient solutions of various composition. By repeated subculture ROBBINS (16) obtained a decreasing growth rate for root tips which suggested that unknown material not present in the nutrient might be essential for continued growth. Although GAUTHERET (7) learned much about nutrient requirements by attempting to grow cambium from large trees, WHITE (18, 20, 21)

measured growth of excised root tips and used new information from nutrition and biochemistry to develop a synthetic nutrient for the continued growth of excised roots. This done, he was able to culture excised stem tissues (22). On the surface of an agar medium the latter grew in an amorphous, undifferentiated state, and by periodic subculture to fresh nutrient they were shown to possess an unlimited capacity for growth. Detailed historical reviews have been adequately given by WHITE (19, 23, 24).

Further investigation of the conditions necessary for growth of plant tissue cultures was undertaken by HILDEBRANDT *et al.* (9, 10). In order to compare various treatments, they cut cultures into pieces approximately 20–30 mg. in weight, allowed them to grow for 6-week periods, and determined the final fresh weights. Through the use of sufficient tissues and replications and statistical analysis of the data, they were able to work out the optimal temperatures, pH, and sucrose concentrations (9) and the optimal concentrations of the other constituents of a nutrient for tobacco and of one for sunflower callus cultures (10). Their methods and results were necessary groundwork to further research involving the culture of excised plant tissues.

Thus far work by various investigators has resulted in (a) obtaining plant tissues which will grow in culture and (b) determining the optimal conditions for their growth. Now that these problems have been partially solved, more attention can be directed to investigation of the nature of the growth process itself in cultures of excised plant tissue. The experiment described here concerns the mechanism of increase in size of a tobacco callus culture and its rate of growth.

Material and methods

The tissues used were 6-week-old subcultures generously supplied by Dr. A. C. HILDEBRANDT from a stock culture which he obtained in 1941 from Dr. P. R. WHITE. WHITE has described (18) the isolation of the original cultures from fragments excised from a region about 5 cm. from the stem tip of *Nicotiana glauca* Grah. ♀ × *N. langsdorffii* Weinm. ♂. Of the original explants, only those which actually contained cambial tissue continued to grow.

The nutrient used was the modification by HILDEBRANDT *et al.* (10) of WHITE's semisolid medium for tobacco callus tissue, the composition of which was kindly imparted by Dr. HILDEBRANDT prior to publication of their report. Salts and sucrose were reagent grade, and redistilled water was used in preparing the nutrient. Difco brand "Bacto Agar" was used at 0.5% concentration. Experimental cultures were grown in the dark in 125-ml. Erlenmeyer flasks containing 50 ml. of medium. For the first 3 weeks they were in an incubator at $26^{\circ} \pm 1.0^{\circ}$ C. and were then transferred, because of approaching summer, to a thermostatically controlled refrigeration unit equipped with a heater operating at this temperature.

Tissues were cut into uniform cubes by means of a dicer consisting of a set of razor blades separated by flat washers carefully measured with a micrometer caliper and held together with small bolts. Two such instruments were made; one had a distance between blades of 1.7 mm., and the other of 2.7 mm. For practice a block of potato was cut into slices, the slices into shoestrings, and the shoestrings into cubes. The weights to the nearest 0.1 mg. of the first thirty cubes of potato picked at random from

each group are shown in table 1. The lesser variability of the larger cubes can be ascribed to variation in the distance between blades during the cutting process; these variations were relatively greater in the smaller cubes.

EXPERIMENTAL

Fifteen tobacco cultures 10–15 mm. in diameter were cut into cubes with dicer A, and ten cultures with dicer B; within each size group all cubes were mixed. Each culture flask was then inoculated with four cubes and was placed in the culture chamber. After contaminated cultures had been eliminated, 168 cubes remained in group A, 71 in group B. At weekly intervals four or five culture flasks in each group were removed; the cultures were rinsed in water, drained on moist filter paper in a covered Petri dish in order to prevent drying, weighed individually, and preserved in formalin-acetic acid-alcohol. Later, in order to determine the sequence of events during the early stages in growth of a cube, additional cultures were cut with dicer A and placed under the same experimental conditions. These cultures were removed at 2-day intervals over a 12-day period and were preserved in Navashin's solution.

Results

MORPHOLOGY AND HISTOLOGY

Growth of the cultures from small cubes during the course of 9 weeks is illustrated in figures 1–4 and is represented diagrammatically in figure 16, which shows how the cultures changed from cubes into somewhat flattened hemispheres.

When a tissue cube was placed on the nutrient medium, the first divisions of cells occurred by the fourth day (fig. 9).

All stages of mitosis were found in the upper portion of the cube to a depth of about seven cells. Further divisions resulted in the proliferation of rows of cells (figs. 5, 10), which became organized into knob-like protuberances (figs. 3, 5, 6).

By the end of the second week the cubes had visibly changed in appearance, the exposed surfaces being covered with small knobs, or bumps, about 1 mm. or less in diameter. As the cultures grew larger, these knobs increased in number.

TABLE 1

Dicer	Distance between blades (mm.)	Range (mg.)	Mean (mg.)	Standard deviation	Coefficient of variability (%)
A...	1.7	4.0–6.0	5.23	0.521	10.0
B...	2.7	18.0–22.0	19.60	0.732	3.7

On a 4-week-old culture they were rounded and more or less regularly spaced (fig. 3); later the knobs were crowded together as if in competition for the surface available (fig. 4). As the cultures grew still older, the knobs often became obscured by the presence of dead cells, which gave to the surface a white, granular appearance.

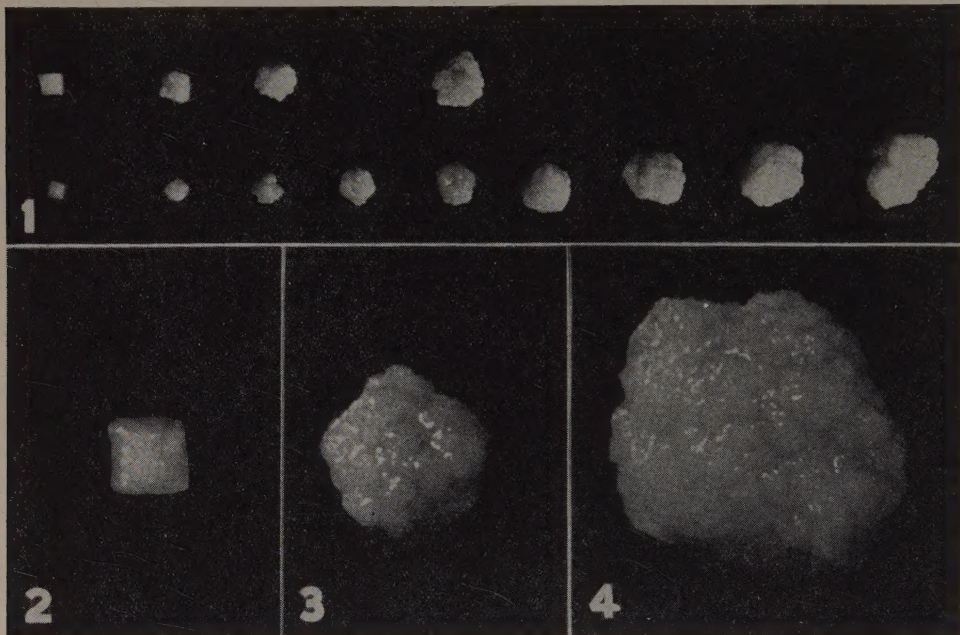
Sections through cultures from successive weeks in the growing period indicated that the knobs increased in number in two ways. One method was by a process of cleavage (fig. 7), which appeared to be the result of failure by a cell, or a row of cells, at the surface to continue dividing. Instead the cell (or cells) enlarged greatly, while other cells at the sides continued to proliferate. The other method was by the formation of new knobs in the subsurface of existing ones (figs. 11, 13).

Growth of a knob was accomplished by divisions of cells at and near the sur-

face (figs. 6, 12). In these divisions new walls were laid down tangentially to the surface. The newly formed cells toward the center of the tissue mass increased in size and, in so doing, pushed the actively dividing cells out along a radius. In this manner a knob continued to grow, its cells forming in orderly rows (figs. 5, 6). Not all divisions within the knob, how-

crushed cell material stained deep red with safranin. Other cells from adjacent knobs formed such intimate contact that, when a culture was snapped in two mechanically, very often the split occurred through knobs rather than around them.

That proliferation from a cube occurred only from the upper portions sug-



FIGS. 1-4.—Largest cultures. Fig. 1, each weekly series; left to right, 0, 2, 3, 4, 5, 6, 7, 8, and 9 weeks. $\times 1$. Fig. 2, freshly cut cube. Fig. 3, fourth week. Knobs on culture are evenly spaced. Fig. 4, eighth week. Knobs appear crowded. Figs. 2, 3, and 4, $\times 5$.

ever, occurred tangentially. As examination of figures 6 and 12 shows, the knobs increased not only in length but in diameter as well. This was effected by cell divisions which occasionally occurred radially (figs. 6, 10, 12) and generally in the center of the knob. New rows of cells were thus formed, which account for the fan-shaped appearance of the knobs in radial section. As the knobs grew, they became appressed laterally, and some of the surface cells in the area of contact were crushed flat (figs. 6, 12). Such

gests that some factor is important in determining where regeneration will occur. A meniscus of liquid nutrient reaching to the top edge formed around the cube soon after it was placed on the agar medium. Thus, a low supply of oxygen may have been effective in limiting growth in the lower portions of the culture. Whether polarity plays a role in this response, however, either partially or even entirely, must await future investigation.

Examination of cross sections of cul-

tures indicated, despite equal amounts of growth, that not all the sub-surface meristems were in an active state of division at the same time (fig. 6). This raises the question as to whether the knobs grew continuously or in an intermittent and possibly rhythmic fashion. No information is available on this point.

DIFFERENCES AMONG CULTURES OF SAME AGE

The growing cubes, though uniform in initial size, varied considerably in weight at each sampling (table 1). Attempts to detect a relationship between histo-

logical aspects and size to account for these variations in amount of growth were not very successful. Certain differences among cultures were noted, however, which might provide clues for an explanation of differences in size and which may form a possible basis for further work.

Differences among cultures at any one time were found in their staining reactions. In some cultures the cell walls in the area approximating the original cube (i.e., in the portion remaining which did not proliferate) were stained deeply red with safranin, although the

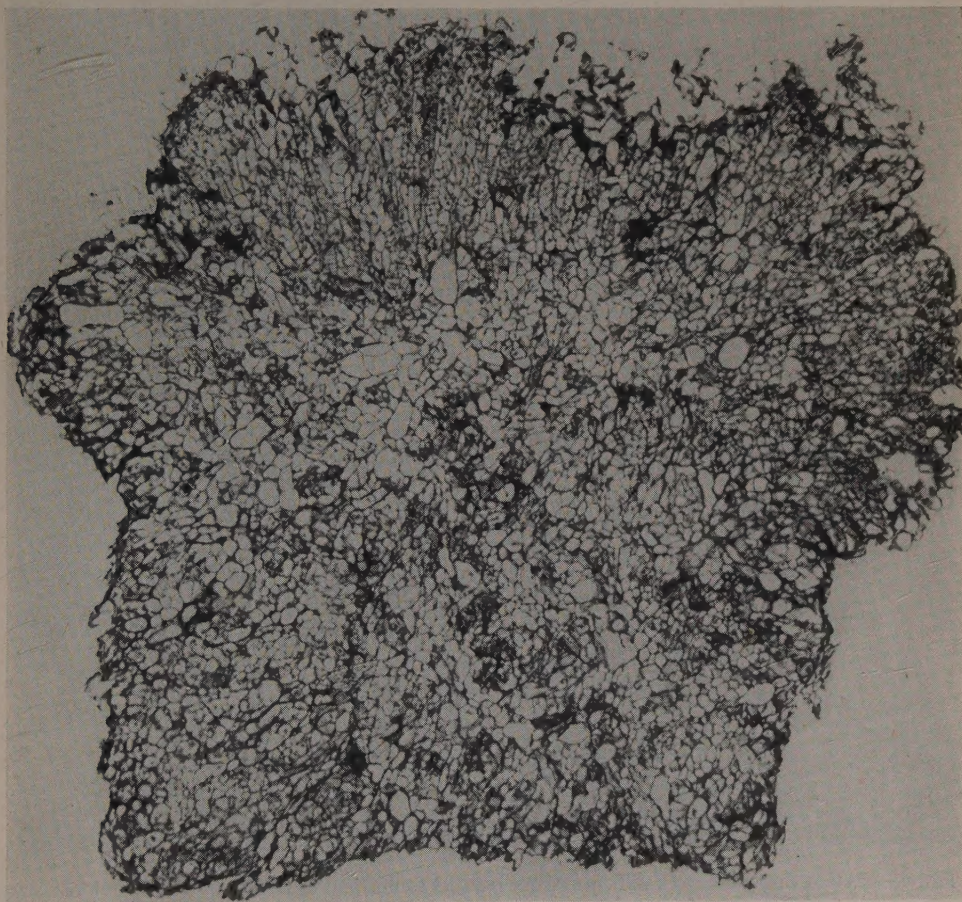


FIG. 5.—Section through 3-week-old culture cut perpendicular to nutrient agar surface, showing that proliferations on culture consist of cells which have been laid down in rows.

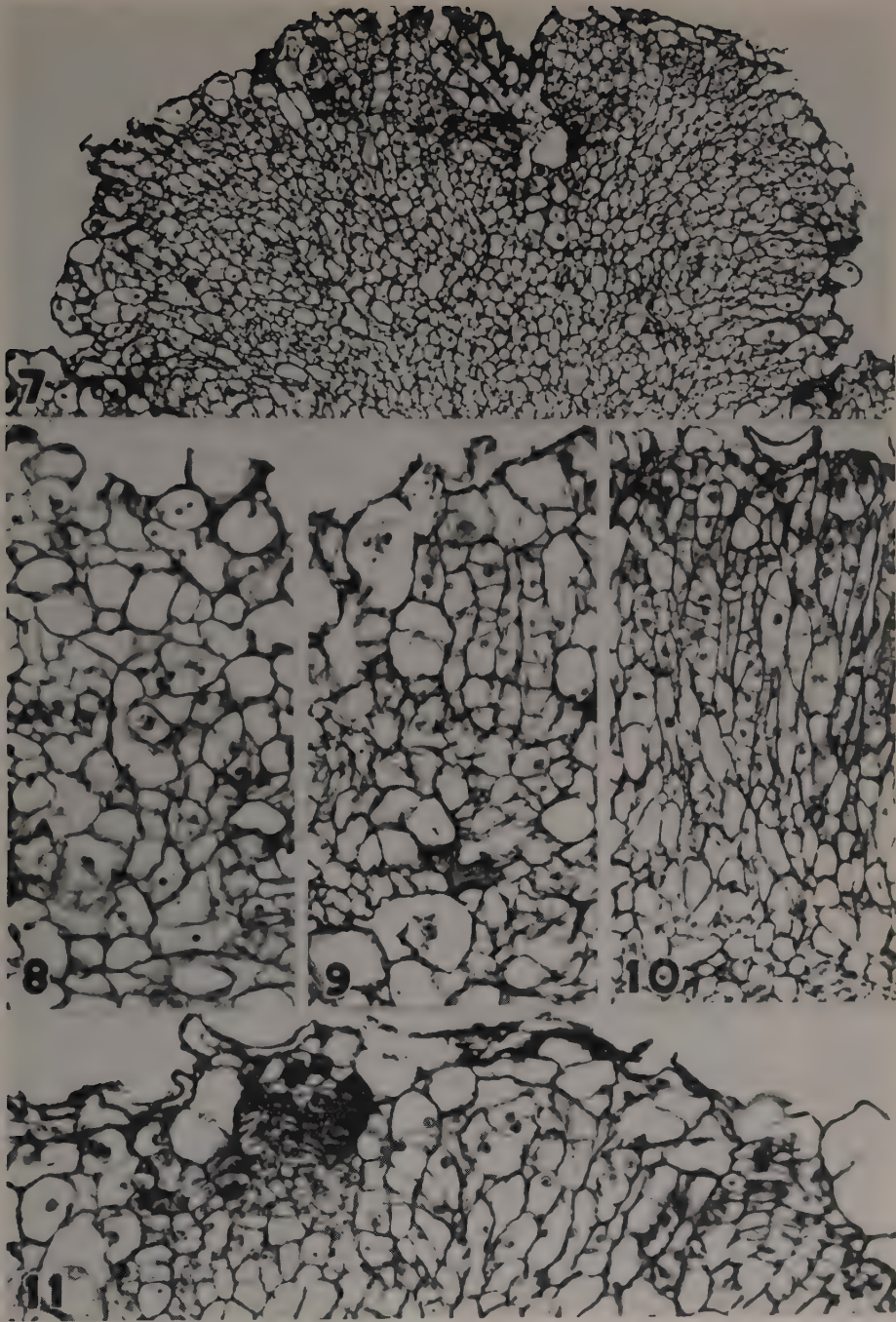
walls of the newly proliferated cells were not. This condition was found in large as well as in small cultures of the same age.

Sections through some of the larger tissue masses in the older age groups showed scattered, apparently dormant, meristematic areas or patches (fig. 14), although the exact way in which they were formed is not clear. Possibly they were cell groups which failed to enlarge after their formation, retaining a meriste-

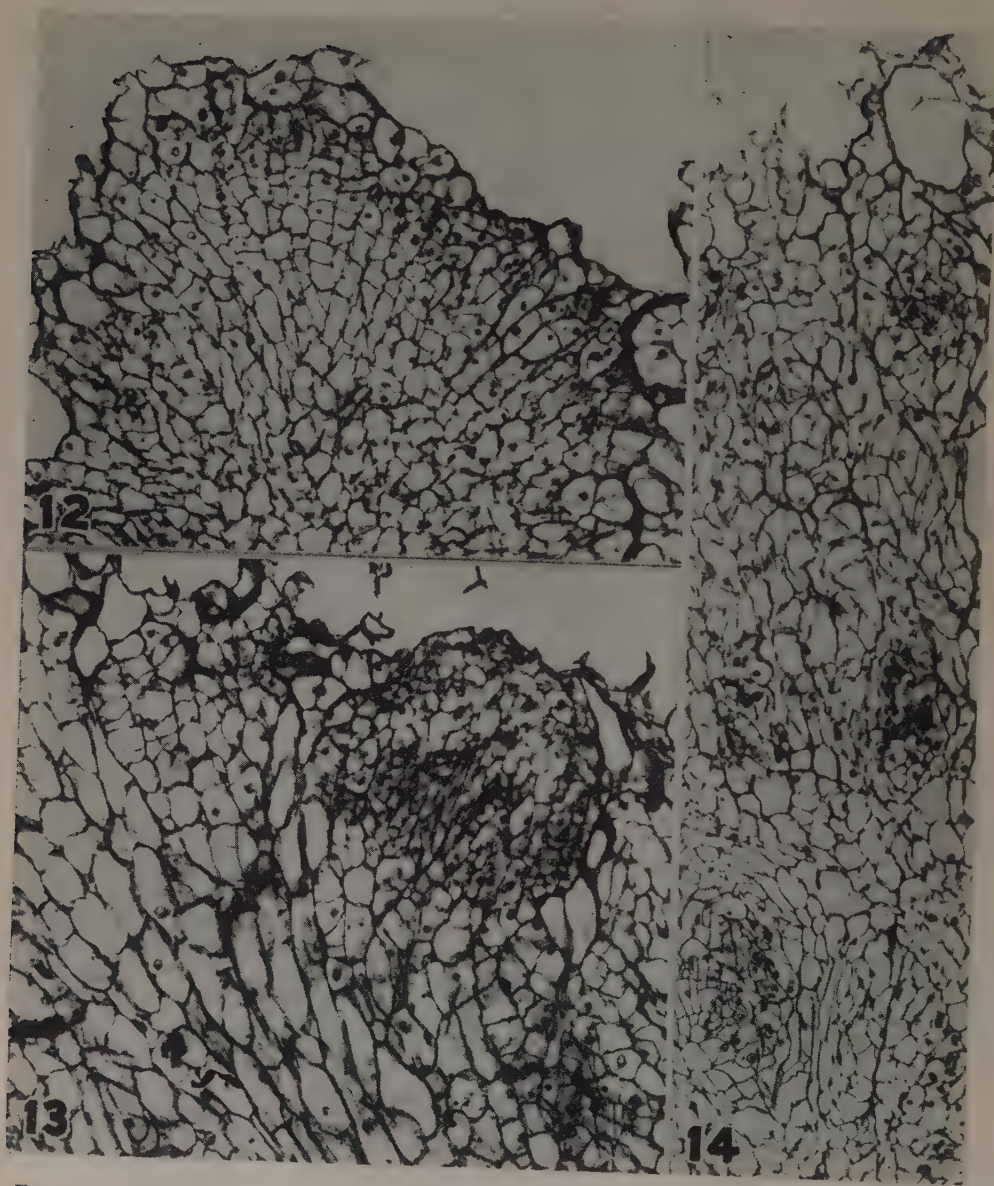
matic appearance. However, a number of the larger patches seemed to be somewhat active. Mitotic figures found occasionally within these areas indicated that new cells were being formed, and the crushing of a number of cells around these areas indicated that enlargement of some of them was also occurring (fig. 14). Little increase in the weight of a culture, however, can be attributed to the formation of these groups of meristematic cells; if these regions had been



FIG. 6.—Section through 6-week-old culture cut parallel to nutrient agar surface (one-fourth of section shown; lines drawn indicate portion remaining of cube from which culture grew). Orderly growth has resulted from meristematic activity at surface and subsurface of culture. Knob at upper right is in active state of division (see also fig. 12).



FIGS. 7-11.—Fig. 7, knob undergoing cleavage to form two knobs. This process appears to be initiated by failure of one or more of dividing cells in subsurface of growing knob to continue dividing. Instead it enlarges greatly, while neighboring cells continue to divide and grow around it. Figs. 8-10, sections through cultures of different age. Fig. 8, freshly cut cube. Fig. 9, 4 days old; cell divisions tangential to surface have resulted in formation of cells in rows. Fig. 10, 12 days old; continued cell division has produced outgrowth; radial divisions have formed new rows of cells. Fig. 11, new meristematic knob forming in subsurface of older knob.



FIGS. 12-14.—Fig. 12, knob showing cell divisions at and near surface. By continued division and enlargement of cells, structures such as this continue to push out, as shown in figs. 5 and 6. Fig. 13, young knob formed below surface and rupturing tissue of old knob outside it. Fig. 14, section through culture (from ninth week) having a somewhat less orderly histological pattern than culture shown in fig. 6. Organized rows of generally mature parenchyma cells are broken up by patches of meristematic cells formed by continued division of some cells deep in culture. Note crushing of adjacent cells around lower patch.

growing actively enough to contribute appreciably to the increase in weight, breaks and tears, which were not evident, would have been found. Some sections through large, older cultures had a bandlike formation of meristematic cells located along the side of a knob where adjacent knobs were in contact. Of such adjacent knobs the side of only one was found to be meristematic.

The cultures were growing in the dark

and were therefore dependent on the medium for carbohydrate. The larger cultures in a given age group were found to contain starch; the smallest ones, however, were found to be free of starch. The areas containing starch were located near the surface in the same general position as the subsurface meristematic cells. In these areas cells with mitotic figures were occasionally found among cells containing large

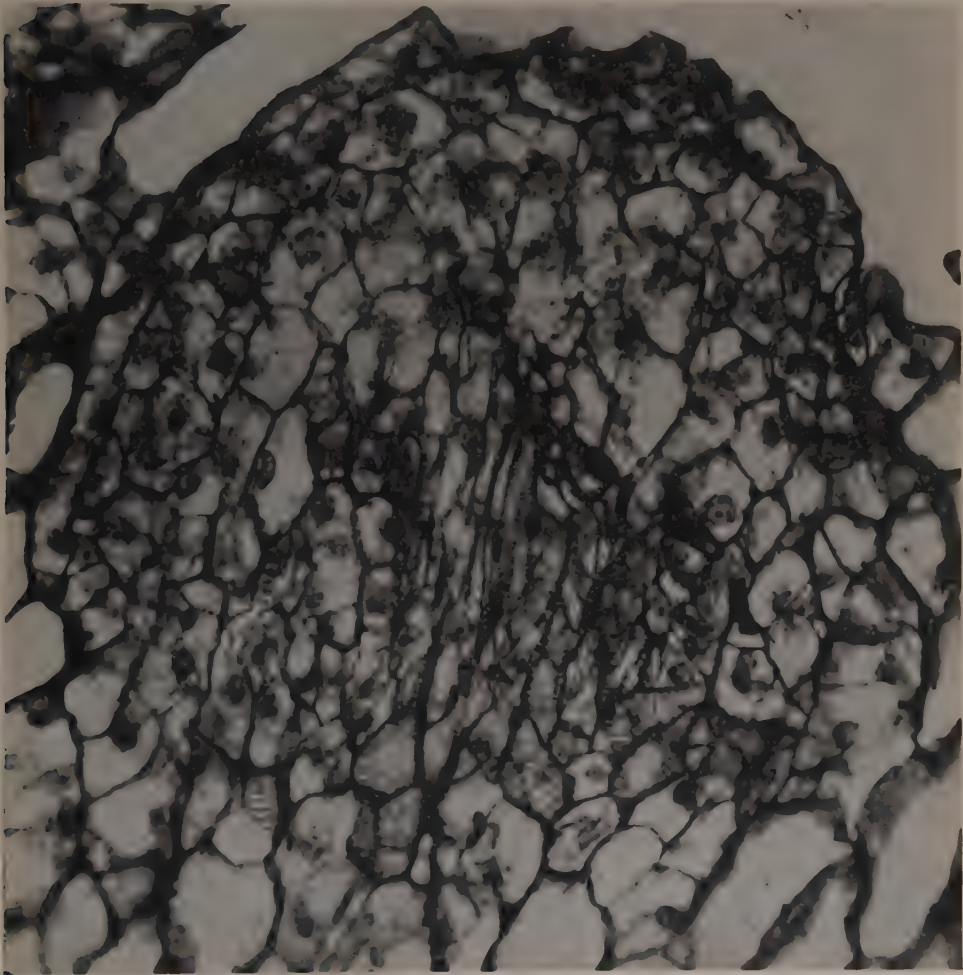


FIG. 15.—Enlargement of young knob in fig. 13, showing "wound tracheids" in center. From serial sections there was no indication of connection between these groups of cells and other similar groups occurring elsewhere in the culture.

starch grains, which is not the normal condition in the apical growing region of angiosperms. It remains to be seen what is the causal connection, if any, between the ability to deposit starch and the greater growth of the large cultures.

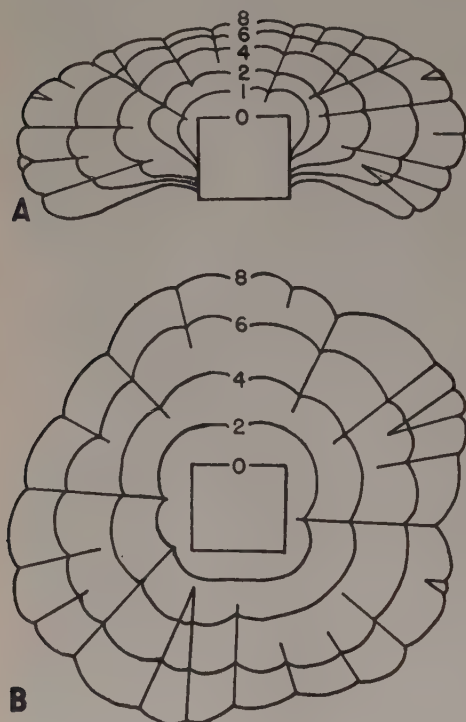


FIG. 16.—Diagrammatic sections through culture illustrating morphological changes during 8 weeks' growth. *A*, section perpendicular to nutrient agar surface; *B*, section parallel to nutrient agar surface. Cubes changed into somewhat flattened hemispheres by growth of knobs in which proliferation of cells occurred in and near surface. Concentric lines represent shape of culture after indicated number of weeks' growth. Radial lines indicate surface of contact between adjacent knobs. Points of radial lines nearest center are points at which knobs have divided. Where two lines radiate from such points, new knobs have been formed.

DIFFERENCES FROM WHITE'S CULTURES

The tobacco cultures first isolated by WHITE grew in a much less organized manner than those here described. A sec-

tion through an 8-week-old culture illustrated by WHITE (22) shows a relatively uniform central area surrounded by loosely organized groupings of meristematic knobs generally separated from one another by groups or rows of much larger cells. Sections through our cultures show, however, much more evidence of growth in an orderly fashion, whereas growth in WHITE's original cultures seems to be more randomized. The reasons for these histological differences are not clear. The fact that WHITE's cultures grew in the light rather than the dark may, however, be a factor.

Another difference between the two sets of cultures concerned the presence of differentiated cells of the wound tracheid type. Whereas in WHITE's cultures these were present as "isolated" cells, here serial sections indicated that at least for short distances many of these cells were connected together and similarly oriented. Figure 15 indicates that the formation of these cells may occur very early in the development of a newly formed meristematic knob.

WEIGHTS OF CULTURES

The weights of the cultures increased with time, but the increase of the largest culture from each weekly sample, in both the *A* and the *B* groups, was much greater than the average increase for each group respectively (table 3). In group *A* after 9 weeks there had been an 18-fold increase in mean weight, while the largest cultures showed a 34-fold increase; in group *B* after 5 weeks there had been a 4.8-fold increase in the mean, and a 6.1-fold increase in the largest cultures. This variance also increases with time as the cultures grow. Only three cubes in group *A* and one cube in group *B* failed to grow at all.

When the weights of the largest cul-

tures from each weekly group sample were plotted, it was discovered that they fell in regular logarithmic relationship to one another. This indicates not only that growth was proportionately progressive but that the largest culture in each weekly sample of twenty cultures had the *maximum* growth rate in its group; it also had the *same* growth rate as the largest culture from each successive weekly sample. Each point of each growth curve in figure 17 represents, therefore, the growth attained by a single culture of maximum growth rate under the conditions of the experiment. The largest cultures in successive weeks in the A and B series are shown in figure 1. The growth curve of the A cultures shows an anomalous weight at 2 weeks, which at present cannot be accounted for except by assuming that the particular culture up to that time had a growth rate the same as the largest cultures in the other weeks and that its initial weight (which was not measured) was approximately 20% greater than the largest measured initial weight at time of inoculation.

Discussion

TISSUE GROWTH AND COMPOUND-INTEREST LAW

BLACKMAN (1) has interpreted the addition of new material by a plant in terms of the compound-interest law. The equation which BLACKMAN found applicable for the growth of an annual plant is

$$W_1 = W_0 e^{rt},$$

where W_1 is the final weight, W_0 is the initial weight, e is the base of natural logarithms, r is the rate of interest, and t is the time. The rate of interest is expressed as percentage increase per day. It is considered by FISHER (3) not only

to be a valuable index for comparing the efficiency of different plants over comparable times and phases of development but as the correct measure for the mean value of the relative growth rate over any period, long or short. For a constant growth rate during a time interval this would be a way of describing numerically the relative rate of growth; for a fluctuating or changing rate of growth during the time interval, such as the alternating growth rate in day and night or a change in average daily

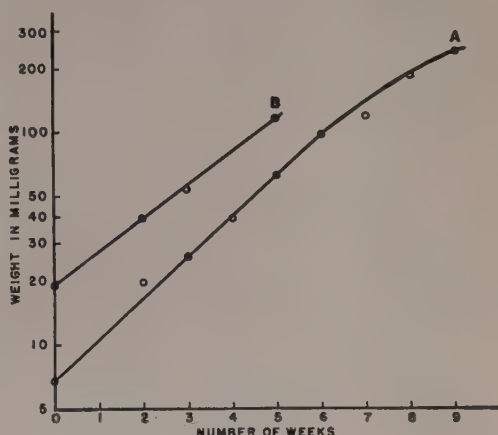


FIG. 17.—Log of wet weight in relation to time of largest cultures in A and B series.

growth rate during a growing period, this would represent an average relative rate of growth for the period. BLACKMAN found that rates of interest for a number of plants, calculated from the data of other workers, ranged from 10.6 to 20.5% per day.

The use of BLACKMAN's formula appears to be particularly applicable for describing the growth of the writer's tissue cultures, since under the easily maintained uniform conditions of the experiment the weights of the largest cultures appear for the first 5 or 6 weeks to indicate constant exponential growth.

Applying the formula, which is more

convenient in the form $\log_e (W_1/W_0) = rt$, r may be calculated as shown in table 2.

The relative growth rates of 6.3% and 5.15% per day represent the maximum rates of growth of the cultures of different initial size under the conditions of the experiment. It should be noted here that the tissues were not subcultured weekly but were growing continuously in one place on the nutritive medium.

Thus far little attention has been given to the quantitative interpretation of the growth rates of plant tissue cultures.

TABLE 2

Group	No. of days (t)	Relative increase	r
A.....	42	14.2	0.063
B.....	35	6.1	0.0515

However, some data showing weight increase of tissues in a given time are available. NOBECOURT (14) reported that a 1-mg. fragment of carrot root tissue increased to 185 mg. in 5 months, with a transfer to fresh nutrient after the first 3 months. The rate of increase calculated from BLACKMAN's formula was 3.48% per day. GAUTHERET (5) measured the increase in weight during the eighth monthly passage of eight pieces of carrot root tissue. These varied in size at the beginning of the culture period from 50 to 180 mg. Average rates of increase for 58 days ranged from 2.8 to 4.9% per day. Data on three tissues from the ninth passage were also given. In 18 days the most rapidly growing culture grew from 125 mg. to 445 mg.—an increase of 7.05% per day. Later he reported that a 0.5-mg. fragment of carrot tissue increased to 15 gm. in a period of 10 months—an increase of 30,000 times. This represents an increase of 3.4% per day. Since nothing is known in these

cases concerning the growth curves during the culture period, it is assumed that these were the average growth rates and that the maximum rates of growth may have been greater. However, the calculated figures represent the maximum rates of growth if the rates of increase were constant.

In his paper describing the first successful culture of *Nicotiana* callus, WHITE (22) reported an average 3-fold increase per passage of 7 days. No figures were cited. Growth on this basis is a rate of increase of 15% per day, a figure comparable with that for the relative growth of an intact plant. Additional information concerning growth rates in plant tissue cultures must await further investigation.

VARIATION WITHIN GROUPS IN GROWTH RATES OF CULTURES

Table 3 shows that weights of cultures in groups A and B increased progressively in variability as the experiment proceeded. Initially all the cultures within each group were approximately the same size and shape. Had they been growing at the same rate, one would, at the end of a growing period, expect the variability of the cultures—expressed in standard deviation—to increase only in proportion to the increase in weight. The coefficient of variability would therefore have remained constant. This, however, was not the case. As the cultures increased in age, their variability increased considerably in both groups A and B. This effect was so pronounced that by the end of the eighth week the weight ratio of the largest to the smallest culture in group A was 10:1.

This increase in variability may be accounted for by either one of the following hypotheses: (a) the cultures were all growing at different rates or (b) they were all growing at the same rates but

different cultures stopped growing at different times after the beginning of the experiment.

Work on this phase of the problem is at present in a preliminary stage. Mention may be made, however, of an experiment in which tobacco callus cultures having an initial weight of 9 mg. or less were grown and weighed individually at weekly intervals during the growing period. The data collected seem to indicate that (a) is the correct hypothesis. Of fourteen pieces cultured for 11 weeks, none had stopped growing during that time. All cultures had increased progressively in weight. At the end of 11 weeks the ratio of the largest to the smallest culture in the group was as 2088:210 mg., or 10:1. At the beginning of the experiment these cultures had weighed 8.8 mg. and 8.4 mg., respectively. Of these, one culture had increased 238 times, the other only 25 times. It seems, therefore, that small cultures of this tobacco callus possess inherent differences which are independent of initial size.

On the basis of these observations the differences in growth rates of initially small cultures must be due to inherent differences in the tissue cubes at the time that they were cut. To what extent these differences between tissue cubes are associated with their original sites or orientation in the tissue mass when cut, or with the growth rate of the culture from which they were subcultured, or to what extent the age of a culture is a factor in regulating subsequent growth rates, are questions yet to be investigated.

DIFFERENCES IN GROWTH RATE IN
RELATION TO INITIAL SIZE
OF EXPLANT

Examination of figure 17 indicates that the growth curves do not have the

same slope. Cultures of larger initial size (B) have a smaller relative growth rate. The difference between the two slopes is 1.15% ($P = < 0.005$ for $N = 6$). Although this percentage may seem small, such a difference can have a profound effect on the final weight. As BLACKMAN

TABLE 3
GROWTH OF UNIFORMLY CUT CULTURES OF
TOBACCO CALLUS

No. of weeks' growth	No. of cultures removed	Weight of largest culture (mg.)	Mean weight (mg.)	Standard deviation	Coefficient of variability (%)
A cultures					
0.....	20	6.8	5.8	0.61	10.5
2.....	20	19.9	10.6	3.28	31.0
3.....	20	26.0	17.6	4.74	26.8
4.....	20	39.0	19.8	6.26	31.7
5.....	20	63.8	36.6	16.80	46.0
6.....	20	96.7	46.4	22.30	48.0
7.....	16	119.6	72.0	27.60	37.8
8.....	16	184.0	93.0	37.50	40.3
9.....	16	231.4	105.0	57.20	54.5
		34×*	18×*	5×*	
B cultures					
0.....	20	19.1	17.2	0.75	4.6
2.....	19	39.7	32.7	3.38	9.4
3.....	16	53.8	43.4	6.27	14.5
5.....	16	116.0	74.4	22.30	30.0
		6.1×†	4.8×†	6.5×†	

* Increase in 9 weeks.

† Increase in 5 weeks.

(1) pointed out, a difference of 1% in relative rates of growth will cause a 2-fold difference in final weight at the end of 69 days, when the rates of growth have remained constant. This suggests that cultures of still smaller initial weight and possessing the same

physiological potentialities would show an even greater relative growth rate.

GAUTHERET (6) has presented evidence which supports this point of view. Using cultures of carrot root tissue, he found that the ratio of the final to the initial weight of a culture varied inversely as the initial weight. From data in his earlier paper (5) on carrot tissues grown for 58 days, it can be shown by arrangement of his data in order of increasing initial weight—50, 50, 110, 115, 150, 155, 160, and 180 mg., with final weights of 585, 735, 1180, 815, 1265, 1270, and 915 mg., respectively—that the relative rates of growth fall into a generally descending series as follows: 4.95, 4.63, 4.10, 3.36, 3.74, 3.62, 3.57, and 2.81%, respectively.

The concept that cultures of smaller initial weight tend to grow relatively more rapidly is further supported by the work of BUCHSBAUM (2) in studies with embryonic cells of the chick. He showed that smaller explants had greater relative increase in 48 hours than larger explants. He attributed this slower growth in larger explants to the inhibiting effect on peripheral cells of the more unfavorably located interior cells, owing to slow diffusion of nutrients and metabolic products.

That these factors operate in plant tissue cultures as well is doubtless true. However, they do not appear to be the complete answer, for, as figure 17 indicates, cultures of the same weight from each of the curves maintained different growth rates for well over 3 weeks. Yet there is every reason to believe that the cultures of maximum growth rate in each group were initially of the same physiological constitution and differed only in size. This difference in growth rate may perhaps be accounted for by the difference in shapes of cultures of similar

weight in the respective groups; at 20 mg., for example, culture B was approaching a hemisphere in shape, while culture A was still a cube.

Summary

1. The growth in darkness of tobacco stem callus tissue on nutrient agar medium was investigated. Growth was studied during a period of 9 weeks in cultures taken from stock subcultures which were 6 weeks old. The experimental cultures were prepared by cutting the stock cultures into uniform cubes of two different sizes by a technique which is described.

2. Increase in size of the cultures resulted from the proliferation of small knoblike protuberances in which division and enlargement of cells was occurring very close to the surface. Knobs increased in number in two ways: (a) by cleavage and (b) by the formation of new growing centers in the subsurface of older knobs. An account of the histology of the growing cultures is presented.

3. Though uniform in initial size and under constant cultural conditions, the cultures within each series grew at different relative rates. Thus there were differences inherent in the initial culture pieces which determined the subsequent rates of growth of the cultures.

4. Over and above the large amount of variation in growth rate of cultures started from pieces of the same size, there were differences in growth rate attributable to the initial size of the explant. The smaller the explant, the greater was the relative growth rate.

5. For each series growth curves were plotted by reference to the largest culture at any time. In these terms the cultures increased exponentially with time for 5–6 weeks. Growth rates are

described in terms of BLACKMAN's formula.

The writer is indebted to the various members of the Department of Botany

for their advice and assistance during the course of the work and in the preparation of the manuscript.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BLACKMAN, V. H. The compound interest law and plant growth. *Ann. Bot.* **33**:353-360. 1919.
2. BUCHSBAUM, RALPH M. Size of explant and volume of medium in tissue culture. *Jour. Exp. Zool.* **63**:483-508. 1932.
3. FISHER, R. A. Some remarks on the methods formulated in a recent article on the quantitative analysis of plant growth. *Ann. App. Biol.* **7**:367-372. 1920.
4. GAUTHERET, R. Sur la possibilité de réaliser la culture indéfinie des tissus de tubercules de carotte. *Compt. Rend. Acad. Sci. Paris* **208**:118-120. 1939.
5. ———. Sur la mesure de la croissance des tissus de carotte cultivés en vitro. *Compt. Rend. Acad. Sci. Paris* **208**:1340. 1939.
6. ———. Sur la culture de tissus de carotte ou de topinambour, même à l'état de lames réduite à une assise de cellules. *Compt. Rend. Acad. Sci. Paris* **214**:805-807. 1942.
7. ———. Titres et travaux scientifiques. Jouve et Cie, Paris. 1942.
8. HABERLANDT, G. Kulturversuche mit isolierten Pflanzenzellen. *Sitzungsber. Akad. Wiss. Wien, math.-naturw. Kl.* **111**:69-92. 1902.
9. HILDEBRANDT, A. C.; RIKER, A. J.; and DUGGAR, B. M. Growth in vitro of excised tobacco and sunflower tissue with different temperatures, hydrogen-ion concentrations and amounts of sugar. *Amer. Jour. Bot.* **32**:357-361. 1945.
10. ———; ———; and ———. The influence of the composition of the medium on growth in vitro of excised tobacco and sunflower tissue cultures. *Amer. Jour. Bot.* **33**:591-597. 1946.
11. KOTTE, W. Wurzelmeristem in Gewebekultur. *Ber. d. bot. Ges.* **40**:269-272. 1922.
12. ———. Kulturversuche mit isolierten Wurzelspitzen. *Beitr. Allg. Bot.* **2**:413-434. 1922.
13. NOBECOURT, P. Sur la pérennité et l'augmentation de volume des cultures de tissus végétaux. *Compt. Rend. Soc. Biol. Paris* **130**:1270. 1939.
14. ———. Cultures de tissus de quelques végétaux. *Bull. Mens. Soc. Linnéenne Lyon* **10**:83-86. 1941.
15. ROBBINS, W. J. Cultivation of excised root tips and stem tips under sterile conditions. *BOT. GAZ.* **73**:376-390. 1922.
16. ———. Effect of autolized yeast and peptone on growth of excised corn root tips in the dark. *BOT. GAZ.* **74**:59-79. 1922.
17. ROBBINS, W. J., and MANEVAL, W. E. Further experiments on growth of excised root tips under sterile conditions. *BOT. GAZ.* **76**:274-287. 1923.
18. WHITE, P. R. Potentially unlimited growth of excised tomato root tips in a liquid medium. *Plant Physiol.* **9**:585-600. 1934.
19. ———. Plant tissue cultures. *Bot. Rev.* **2**:419-437. 1936.
20. ———. Amino acids in the nutrition of excised tomato roots. *Plant Physiol.* **12**:793-802. 1937.
21. ———. Glycine in the nutrition of excised tomato roots. *Plant Physiol.* **14**:527-538. 1939.
22. ———. Potentially unlimited growth of excised plant callus in an artificial nutrient. *Amer. Jour. Bot.* **26**:59-64. 1939.
23. ———. A handbook of plant tissue culture. Jaques Cattell Press, Lancaster, Pa. 1943.
24. ———. Plant tissue cultures. II. *Bot. Rev.* **12**:521-529. 1946.

